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14. ABSTRACT
In this proposal, we are testing the hypothesis that the risk of developing prostate cancer and the aggressiveness of the disease are influenced by protein glycosylation. We postulate that glycobiology contributes to the higher susceptibility of African American men. The study population is assembled and an annotated sample repository is ready for use. Methods for the glycomic analysis of fractionated proteins are in place. We have evaluated mRNA datasets comparing prostate cancer in African American and Caucasian men. The analysis finds some connections to glycosylation. We have now analyzed pooled and unpooled samples of men by optimized analyses of fractionated serum in order to evaluate the glycomic changes in prostate cancer health disparity. The analysis and the interpretation of results is ongoing.

15. SUBJECT TERMS
Prostate cancer, glycomic analysis, health disparity

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Table of Contents

Page

Progress Report, W81XWH-09-1-0202, Glycomic Analysis of Prostate Cancer, 10. 2011

INTRODUCTION

In this proposal, we are testing the hypothesis that the risk of developing prostate cancer and the aggressiveness of the disease are influenced by protein glycosylation. We postulate that glycobiology contributes to the higher susceptibility of African American men. A major goal of the study is to evaluate the glycosylation differences in prostate cancer of African American and Caucasian men living in the Baltimore-Washington metropolitan area.

Aim1. Quantify N-glycans in serum of men in a case-control study of prostate cancer with a focus on differences between Caucasians and African Americans.

Aim2. Evaluate prediction accuracy of select N-glycans for the detection of prostate cancer.

Aim3. Perform an exploratory study of N-glycans in urine of the participants and correlation of glycans with gene expression in existing array datasets.

BODY

We have now assembled a study of protein glycosylation which includes comparison of healthy a biopsy controls with cancer cases. We analyze both pooled samples and individual patient samples in a pooled-unpooled study design **(Table 1)**.

All the pooled samples were analyzed and we are completing the analysis of the individual samples. Because we have found that the N-glycosylation of serum proteins is strongly affected by the glycosylation of immunoglobulins **(Figure 1)**, we have separated each serum sample into three layers (liver secreted proteins, IgG and other immunoglobulin layers). The separation of immunoglobulins was carried out on protein A and G microcolumns. Glycosylation of enzymatically detached glycans in each protein layer has been quantified by mass spectrometric analysis of the permethylated N-glycans. This will allow us to analyze separately the N-glycans associated with immunoglobulins (immune system related glycosylation) and remaining proteins (non-immune glycosylation response).

As a first study, we have completed the analysis of the enzymatically detached N-glycans in repeatedly sampled samples of 20 healthy controls (50% African American). For each of the participants, we have obtained four blood samples in the span of six months. Overall, we have identified consistently 85 N-glycans in the liver secreted fraction, and 51 glycans in each of the IgG and other immunoglobulin layers. The within subject variability was 16-24%. CVs were positively correlated with glycan mass and inversely correlated with intensity and tended to be higher among glycan structures that were fucosylated rather than sialylated. We have found that age, race and gender had a smaller effect on glycan measurement than lifestyle factors such as body mass index (BMI), use of non-steroidal anti-inflammatory drugs (NSAIDs), smoking status and education **(Figure 2)**. These factors should be accounted for in the study of N-glycans in the progression of various diseases.

The analysis of 32 pooled samples **(Table 1)** showed that differences related to immunoglobulin G exist between AA and CA men and are more apparent than those in the liver secreted proteins **(Figure 3)**. These differences could potentially explain differences in susceptibility to prostate cancer and will be further explored in the individual patient samples.

In addition, we have begun a comparative analysis of gene expression in African American (AA) and European American (EA) men in order to identify differentially expressed glycogenes. Glycogenes were defined as probes selected by the Consortium for Functional Glycomics (CFG) for inclusion on their mRNA array (http://www.functionalglycomics.org/static/consortium/resources/resourcecoree.shtml). The array contains probe sets for 1175 unique glycogenes. We looked for intersection of this gene set with the genes observed as differentially abundant in GSE6956 (Wallace et al, 2008, PMID: 18245496) and GSE17356 (Timofeeva et al, 2009, PMID: 19724911). These are the only two mRNA expression studies we identified comparing the expression in prostate cancer of AA and EA men. GSE6956 contains data of 89 samples; prostate tumor (n=69) and non-tumor tissue (n=20). We used the array data of 69 tumor samples for our study. Samples in GSE17356 are primary prostate cancer epithelial cell cultures (n=27). The authors compared the mRNA expression in prostate cancer samples isolated from AA and EA men.

By using the SAMR package and Bayesian regularized t test on re-annotation profile, we identified 28 glyco genes among the differentially expressed mRNA in GSE6956 and 40 glyco genes among the differentially expressed mRNA in GSE6956. We will further evaluate whether the glycosylation related genes identified in our analysis affect prostate cancer disparity and glycan profiles observed in our study. Comparison of mRNA expression in GSE6956 and GSE17356 showed 40 genes consistently up- or down- regulated in both sets (comparison of the AA and EA men). The genes are listed in **Table 2**. Two of the genes belong to the glycogene set **(Table 3)**. A strong association was found between the dysregulated genes and the insulin regulation of fatty acid metabolism.

KEY RESEARCH ACCOMPLISHMENTS

1. The study population is assembled and an annotated sample repository is ready for use.
2. Methods for the glycomic analysis of fractionated proteins are established
3. Separation of immunoglobulin associated glycans improves glycomic analyses
4. mRNA array informatics show 40 genes differentially abundant consistently in two existing datasets but these are the only two datasets comparing African American and Caucasian men we identified
5. Analysis of 20 healthy controls with samples acquired at four different times is completed
6. The analysis of glycans in 32 pooled samples (Table 1. below) is completed and the analysis in individual samples of 142 men is completed.

REPORTABLE OUTCOMES

Poster "Detection of prostate cancer using glycomic analysis: can differences in glycosylation explain the health disparity of the disease"; was presented at the IMPACT meeting, Orlando, FL in March 2011.

One publication is almost ready for submission (summary of the repeated healthy control analysis).

CONCLUSION

The study proceeds along the projected aims. We have established the methods for analysis of glycans in serum fractionated into three layers. We have completed analysis of pooled samples and plan to analyze a sufficient number of samples to evaluate the glycomic changes in prostate cancer health disparity. Completion of the analysis of serum samples, exploratory analysis of urine, and data analysis with preparation of manuscripts are our goals for the final year of the study.

REFERENCES

NA

Appendices

NA

Supporting Materials

Source	Cases		Healthy controls		Biopsy controls	
Race	CA	AA	CA	AA	CA	AA
Sample number	70	70	50	50	38	38
Pool number	7	7	5	5	4	4

Table 1. Study design includes Caucasian American (CA) and African American (AA) men in each category of cancer cases, healthy population controls, and biopsy controls verified to be cancer free. Pools represent groups of 10 or 8 patient samples pooled for an exploratory analysis.

Figure 1. Separation of serum N-glycans into liver secreted and immunoglobulin-derived layers (below)

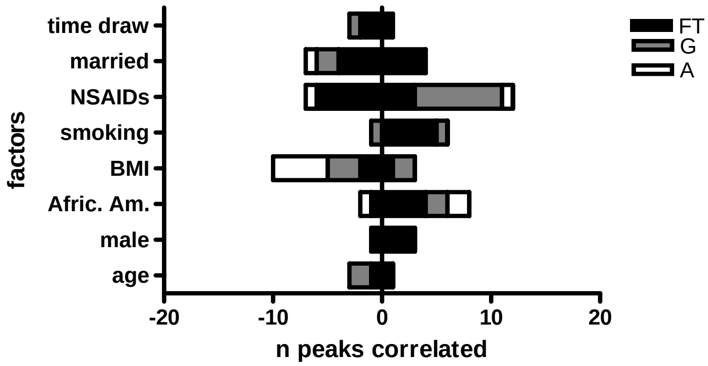
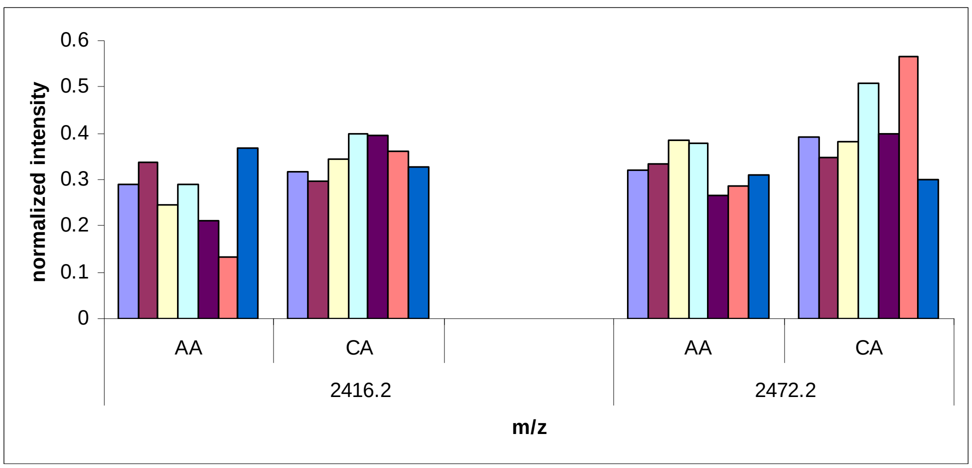


Figure 2. Number of glycans affected by host characteristics

A.



B.

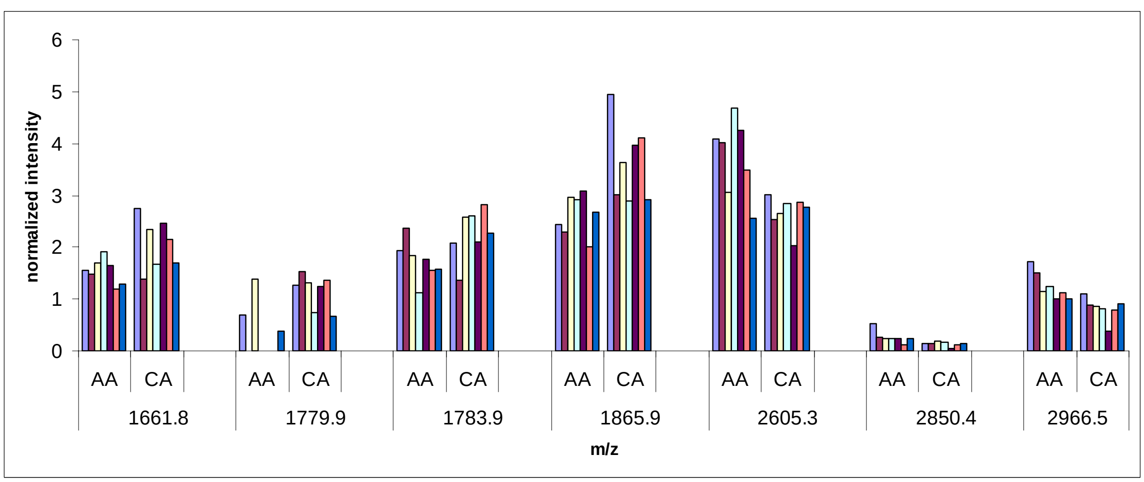


Figure 3. Differences in pooled samples of Caucasian and African American men by glycan mass: A. Liver secreted protein fraction; B. Immunoglobulin G fraction.

Gene symbol	FC_6956	p_6956	FC_17356	p_17356
ADI1	0.65	3.04E-05	0.46	3.04E-05
AMFR	1.68	4.29E-05	3.00	3.01E-07
APIP	0.83	5.49E-03	0.66	3.94E-03
ATP11B	0.83	6.23E-03	0.74	5.34E-03
BIN2	1.17	5.62E-03	1.16	9.55E-03
C14orf108	0.84	3.56E-04	0.72	7.39E-04
C18orf10	1.16	8.56E-04	1.20	7.54E-03
C3orf37	0.90	3.83E-03	0.75	6.45E-04
C7orf49	0.89	2.60E-03	0.80	2.63E-03
CLC	0.91	2.34E-04	0.89	6.06E-03
CNNM4	0.91	8.74E-03	0.80	1.55E-03
CPSF4	0.92	6.05E-03	0.86	5.04E-03
CRYBB2	1.93	7.80E-11	2.20	1.34E-04
CTNNB1	1.37	7.18E-07	2.13	3.91E-07
EBI2	1.55	4.06E-03	1.30	5.57E-03
FAM128A	0.76	1.36E-03	0.63	9.08E-03
FASTKD3	0.86	8.46E-03	0.70	2.99E-03
GOLPH4	1.18	1.33E-03	2.05	7.47E-10
IL20RA	0.71	2.15E-03	0.70	6.00E-04
INDO	1.39	2.17E-03	1.26	5.65E-04
LEPROT	1.19	6.06E-03	1.24	2.59E-03
MAP3K15	1.20	9.75E-06	1.28	8.42E-03
MAPK8	0.89	4.02E-03	1.16	8.05E-04
MGAT1	1.07	8.32E-03	1.19	9.84E-03
MRPL35	0.89	6.66E-03	0.81	8.53E-03
MRPS7	0.89	5.97E-03	0.80	1.70E-03
MTA1	0.89	5.05E-03	1.22	6.64E-03
MXRA7	1.38	7.59E-04	1.35	8.08E-03
NARS2	0.78	2.03E-04	0.75	3.12E-04
PAPD1	0.88	1.29E-04	0.81	4.44E-03
PRPSAP1	0.88	9.89E-04	0.80	4.45E-03
PSPH	2.34	1.70E-09	2.02	9.52E-03
RFX5	1.10	1.53E-03	0.79	3.30E-03
RPP38	0.87	6.80E-04	0.71	8.95E-06
SFXN1	0.88	2.89E-03	0.56	3.49E-03
SOS1	1.27	1.53E-03	1.67	9.72E-07
TTC27	0.91	9.38E-03	0.74	2.70E-03
VPS53	0.92	4.92E-03	1.24	1.06E-03
VRK2	0.88	2.87E-03	0.80	1.50E-03
ZNF227	0.87	1.87E-03	0.81	2.97E-03

Table 2. mRNA expression of 40 genes consistently up- or down- regulated in the comparison of the AA and EA men in both GSE6956 and GSE17356 datasets.

Probe	Gene Symbol	FC_6956	p_6956	FC_17356	p_17356
202377_at	LEPROT	1.19	6.06E-03	1.23	2.59E-03
201126_s_at	MGAT1	1.06	8.32E-03	1.19	9.84E-03

Gene Symbol	Source1	Source2	Name	NM
LEPROT	xGrowth Factors & Receptors	Miscellaneous	LEPROT [leptin receptor gene-related protein]	NM_017526
MGAT1	Glycan-transferase	GlcNAc-T	MGAT1 (mannosyl (alpha-1,3-)-glycoprotein beta-1,2-N-acetylglucosaminyltransferase)	NM_002406

Table 3. Glycogenes selected from Table 2.

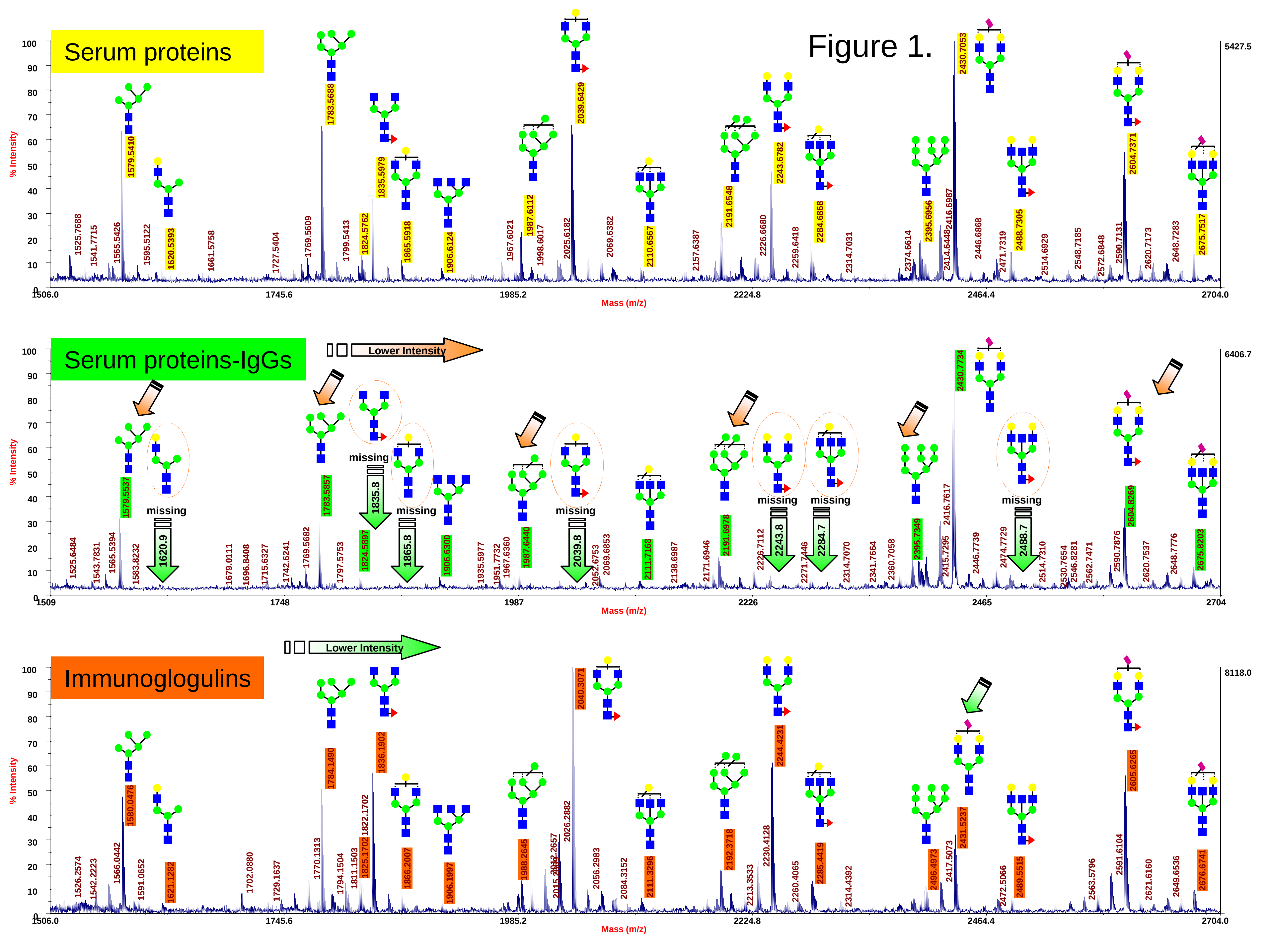


Figure 1.
Serum proteins
Serum proteins-IgGs
Immunoglogulins
Lower Intensity
missing
% Intensity
Mass (m/z)
5427.5
6406.7
8118.0